1. PRENATAL DIAGNOSIS OF CHROMOSOMAL DISORDERS - molecular aspects

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The standard measures for population health outcomes is based on maternal, infant and under five mortality rates. Health care of mother and unborn child is the most important part of population health. Therefore the care for mother and child health during pregnancy and delivery, assessment of all risks during pregnancy is of utmost importance of any health care system.

It is known that perinatal mortality is caused in 20-25 percent of cases by inhaerited anomalies of fetuses and many of theese might be explained by genetic disorders. In general genetic disorder is a condition caused by abnormalities in genes or chromosomes. Chromosomes are complex bodies in cell nucleus as carriers of genes. While some diseases are due to genetic abnormalities acquired in a few cells during life, the term "genetic disease" most commonly refers to diseases present in all cells of the body and present since conception. Some genetic disorders are caused by chromosomal abnormalities due to errors in meiosis, the process which produces reproductive cells such as sperm and eggs. Examples include Down syndrome (extra chromosome 21), Turner Syndrome (45X0) and Klinefelter's syndrome (a male with 2 X chromosomes). Other genetic changes may occur during the production of germ cells by the parent. One example is the triplet expansion repeat mutations which can cause fragile X syndrome or Huntington's disease. Defective genes may also be inherited intact from the parents. In this case, the genetic disorder is known as a hereditary disease. This can often happen unexpectedly when two healthy carriers of a defective recessive gene reproduce.

Chromosomal abnormalities are disruptions in the normal chromosomal content of cell and are a major cause of genetic diseases in humans; some chromosomal abnormalities do not cause disease in carriers such as translocations or chromosomal inversions although they may lead to higher proportions of chromosomal disorder in child. Abnormal number of chromosomes or chromosome sets caled aneuploidy may cause letal condition or give rise in genetic disorders. Furthermore the gain or loss of chromosome material may lead to genetic disorder (deletion, extra copy as trisomy). Chromosomal mutations produce changes in whole chromosomes (more than one gene) or in the number of chromosomes present.

1.1 The major chromosomal abnormalities

The risk for chromosomal abnormalities increases with increasing maternal age, mainly because non-dysfunctional events in meiosis are more likely, and result in trisomies. To make it more complex the “mosaicism” must be added. A "mosaic" is a person with a combination of two cell lines with different karyotypes (normal and abnormal). When karyotyping is performed, multiple cells are analyzed to rule out this possibility. The mosaic condition is not as severe as the completely abnormal karyotype, and the features may not be as marked, and live births may be possible. Sometimes the mosaic’s is confined to the placenta ("confined placental mosaicism").

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A placenta with an abnormal karyotype (confined placental mosaicism) may lead to stillbirth, even though the fetus has a normal karyotype; conversely, a placenta with a normal karyotype may allow longer survival for a fetus with a chromosomal abnormality. Rarely, a translocation of part of one chromosome to another in the parent will be passed on to the child as a partial trisomy (such as 6p+ or 16p+) which may not be as severe as a complete trisomy.

- Trisomy 21 (extra chromosome 21): Down syndrome; incidence based upon maternal age, though translocation type is familial; features can include: epicanthal folds, simian crease, brachycephaly, cardiac defects.
- Trisomy 18 (47, XY,+18): Features include micrognathia, overlapping fingers, horseshoe kidney, rocker bottom feet, cardiac defects, diapragmatic hernia, omphalocele.
- Trisomy 13 (Patau Syndrome also called D-Syndrome): Features include microcephaly, cleft lip and/or palate, polydactyly, cardiac defects, holoprosencephaly.
- Trisomy 16: Seen in abortuses from first trimester. Never liveborn.
- Monosomy X: Turner's syndrome (45,X 0); can survive to adulthood; features include short stature, cystic hygroma of neck (leading to webbing), infertility, coarctation.
- Klinefelter's syndrome (XXY, a male with 2 X chromosomes); features include elongated lower body, gynecomastia, testicular atrophy (incidence: 1/500 males)
- Triploidy: There is often a partial hydatidiform mole of placenta. Fetal features include 3-4 syndactyly, indented nasal bridge, small size.
- Idic 15 or isodicentric 15 :inverted duplication of chromosome 15 or tetrasomy 15
- Jacobsen syndrome also called the terminal 11q deletion disorder. This is a very rare disorder. Those affected have normal intelligence or mild mental retardation, with poor expressive language skills. Most have a bleeding disorder.
- XYY syndorm. XYY boys are usually taller than their siblings. Like XXY boys and XXX girls, they are somewhat more likely to have learning difficulties.
- Triple XXX syndrome. XXX girls tend to be tall and thin. They have a higher incidence of dyslexia.

A host of other chromosomal abnormalities are possible. In general, fetal loss earlier in gestation, and multiple fetal losses, more strongly suggests a possible chromosomal abnormality.

1.2 Prenatal diagnosis

Prenatal diagnosis employs a variety of techniques to determine the health and condition of an unborn fetus. Without knowledge gained by prenatal diagnosis, there could be an untoward outcome for the fetus or the mother or both.

Specifically, prenatal diagnosis is helpful for:

- Managing the remaining weeks of the pregnancy
- Determining the outcome of the pregnancy
- Planning for possible complications with the birth process
- Planning for problems that may occur in the newborn infant
- Deciding whether to continue the pregnancy
- Finding conditions that may affect future pregnancies

There are a variety of non-invasive and invasive techniques available for prenatal diagnosis. Each of them can be applied only during specific time periods during the pregnancy for greatest utility.

Indications for prenatal diagnostic testing include:age of mother, Down syndrome in previous pregnancy or family, structural aberrations in previous pregnancies or in family members, autosomal genopaties, X-linked genetic disorders, neuronal tube defects in previous pregnancies, mental retardation in family (linked to fragile X) present ultrasound suspicion, consanguinity, pathological finding in prenatal serum screening, other reasons (viral infection, radiation).

1.3 Source of samples for prenatal testing

Prenatal diagnosis of chromosomopathies as well as genetic disorders is based on invasive and non-invasive techniques.

Chorionic villi sampling (CVS)In this procedure, a catheter is passed via the vagina through the cervix and into the uterus to the developing placenta under ultrasound guidance. Alternative approaches are transvaginal and transabdominal. The introduction of the catheter allows sampling of cells from the placental chorionic villi. These cells can then be analyzed by a variety of techniques. The most common test employed on cells obtained by CVS is chromosome analysis to determine the karyotype of the fetus. The cells can also be grown in culture for biochemical or molecular biologic analysis. CVS can be safely performed between 9.5 and 12.5 weeks gestation.

CVS has the disadvantage of being an invasive procedure, and it has a small but significant rate of morbidity for the fetus; this loss rate is about 0.5 to 1% higher than for women undergoing amniocentesis. Rarely, CVS can be associated with limb defects in the fetus. The possibility of maternal Rh sensitization is present. There is also the possibility that maternal blood cells in the developing placenta will be sampled instead of fetal cells and confound chromosome analysis. The obtained material is used for fluorescent in situ hybridization (FISC), short tandem repeats (STR) , DNA and some biochemical analyses.

Amniocenthesis (transvaginal aspiration of amnionic fluid 15-20 weeks of pregnancy) is the most used method (risk below 0,5 %) for sample for all kind of analyses.

Preconception – preimplantation diagnosis is possibility applied in connection with in vitro fertilization (IVF) to make diagnosis at the gamete stage or performing the biopsy of one or two blastomeres by aspiration with micropipette. Preimplantation diagnosis is now offered as an alternative to conventional prenatal diagnosis in following cases: recessive or dominant hereditary disorders linked to chromosome X, monogenic disorders of authosomal inheritance (recessive or dominant) and the detection of translocations (couples who are carriers of chromosome abnormality of number or structure).

Maternal blood sampling for fetal blood cells is a new non-invasive technique that makes use of the phenomenon of fetal blood cells gaining access to maternal circulation through the placental villi. Ordinarily, only a very small number of fetal cells enter the maternal circulation in this fashion (not enough to produce a positive Kleihauer-Betke test for fetal-maternal hemorrhage). The fetal cells can be sorted out and analyzed by a variety of techniques to look for particular DNA sequences, but without the risks that these latter two invasive procedures inherently have. Fluorescence in-situ hybridization (FISH) is one technique that can be applied to identify particular chromosomes of the fetal cells recovered from maternal blood and diagnose aneuploid conditions such as the trisomies and monosomy X.

The problem with this technique is that it is difficult to get many fetal blood cells. There may not be enough to reliably determine anomalies of the fetal karyotype or assay for other abnormalities.

1.4 Molecular analysis

The technologies developed for the Human Genome Project, the recent surge of available DNA sequences resulting from it and the increasing pace of gene discoveries and characterization have all contributed to new technical platforms that have enhanced the spectrum of disorders that can be diagnosed prenatal. The importance of determining the disease-causing mutation or the informative ness of linked genetic markers before embarking upon a DNA-based prenatal diagnosis is, however, still emphasized.

Different fluorescence in situ hybridization (FISH) technologies provide increased resolution for the elucidation of structural chromosome abnormalities that cannot be resolved by more conventional cytogenetic analyses, including micro deletion syndromes, cryptic or subtle duplications and translocations, complex rearrangements involving many chromosomes, and marker chromosomes. Interphase FISH and the quantitative fluorescence polymerase chain reaction are efficient tools for the rapid prenatal diagnosis of selected aneuploidies, the latter being considered to be most cost-effective if analyses are performed on a large scale. There is some debate surrounding whether this approach should be employed as an adjunct to karyotyping or whether it should be used as a stand-alone test in selected groups of women.

Interphase and metaphase FISH, either as a single probe analysis, or using multiple chromosome probes, can give reliable results in different clinical situations.

It should be noted that there may be variation in probe signals both between slides (depending on age, quality, etc. of metaphase spreads) and within a slide. Where a deletion or a rearrangement is suspected, the signal on the normal chromosome is the best control of hybridisation efficiency and control probe also provides an internal control for the efficiency of the FISH procedure.

Depending on the sensitivity and specificity of the probe and on the number of cells scored, the possibility of mosaicism should be considered, and comments made where appropriate. By using locus-specific probes at least 5 cells should be scored to confirm or exclude an abnormality. Multiprobe analysis: three cells per probe should be scored to confirm a normal signal pattern. Where an abnormal pattern is detected, confirmation is advisable. In prenatal interphase screening for aneuploidy signals should be countered in at least 30 cells for each probe set. A minimum 100 cells should be scored.

When hybridisation is not optimal, the test should be repeated. When a deletion or another rearrangement is suspected, the results must be confirmed with at least one other probe.

Results should preferably be followed up by karyotype analysis. This is essential when there are discrepancies between the expected laboratory findings, and the clinical referral.

Before introducing interphase FISH as a diagnostic technique, staff need appropriate training on the type of samples to be analysed. Laboratories should set standards for classification of observations and interpretation of results.

More recently new method for fast identification of chromosomal abnormalities has been developed as high resolution array comparative genomic hybridization (aCGH) which provide genome-wide analysis of chromosome copy number and structural change .The chip technology provide investigation of genetic causes associated with dysmorphic features, mental retardation, developmental delay, multiple congenital abnormalities .The commercial chip include more tha 40 abnormalities including duplications and microdeletion regions. It is expected that evaluation of this technique will prove scientifically based evidence for named advantages.

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